

Genetic Status of *Angelica japonica* var. *boninensis* (Umbelliferae) Distributed in the Bonin and the Daito Islands

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Three infraspecific taxa, var. *japonica*, var. *hirsutiflora*, and var. *boninensis*, were recognized in *Angelica japonica* by Yamazaki (1990). He considered that var. *boninensis* is distributed both in the Bonin and the Daito Islands. In this paper, in order to evaluate taxonomic status of var. *boninensis*, we examined the populations of the three varieties on the basis of allozyme analysis. In the obtained UPGMA dendrogram, three major clusters of the populations from the following three areas were recognized: I) the southern part of Kyushu, the Hedo population of Okinawa Island and the Daito Islands; II) the Bonin Islands; and III) Okinawa Island and the Yaeyama Islands. The plant populations of the Daito and the Bonin Islands, which were identified as var. *boninensis* by Yamazaki (1990), were divided into two different clusters based on the UPGMA dendrogram. The values of genetic identities among the three clusters were lower than the mean values of those among conspecific plant populations. Differences in leaflet shape coincided with these clusters. We concluded that the clusters I, II, and III can be taxonomically treated as independent species *Angelica japonica* A. Gray, *A. boninensis* Tuyama, and *A. hirsutiflora* Liu, Chao et Chung, respectively.

Key words: allozyme, *Angelica boninensis*, *Angelica hirsutiflora*, *Angelica japonica*, Bonin Islands, Daito Islands, taxonomy

Angelica japonica A. Gray is a perennial maritime plant distributed in the warm temperate and subtropical zone of Japan and Taiwan (Hiroe & Constance 1958, Kitagawa 1982, Yamazaki 1990). Three infraspecific taxa, var. *japonica* (distributed in Kanto District and westward in Honshu, Shikoku, Kyushu, the Osumi Islands, and the Amami Islands excepting Yoron Island), var. *boninensis* (the Ogasawara and the Daito Islands), and var. *hirsutiflora* (Yoron Island, Okinawa Island and southward) were recognized based on their morphological characteristics shown in Table 1 (Yamazaki 1990).

Seo *et al.* (2004) reported that *Angelica japonica* on the Ryukyu Islands can be divided into two

genetically distinguishable groups. One consists of the populations ranging from the southern part of Honshu, Kyushu, the Osumi Islands, and the Amami Islands (excepting Yoron Island) to the northern part of Okinawa Island, and the other consists of the populations of Yoron Island, Okinawa Island and southward. These two groups corresponded primarily to the var. *japonica* and var. *hirsutiflora*, respectively. However, Seo *et al.* (2004) did not analyze plant materials from the Bonin Islands and the Daito Islands, which were recognized as var. *boninensis* by Yamazaki (1990).

In this paper, in order to evaluate the taxonomic status of var. *boninensis*, we examined genetic relationships among the three infraspecific taxa of

TABLE 1. Discriminating morphological characters among the three varieties of *Angelica japonica* by Yamazaki (1990).

Character	var. <i>japonica</i>	var. <i>hirsutiflora</i>	var. <i>boninensis</i>
Serration of radical and lower cauline leaves	acute	obtuse	obtuse
hair on pedicels ¹	-, +, ++	++	-, +
wings of fruits	thin	thick	thin

¹ -, glabrescent; +, sparse; ++, dense

Angelica japonica, with special reference to the genetic differentiation among the populations in the Bonin Islands, the Daito Islands, and their adjacent areas on the basis of allozyme analysis.

Materials and Methods

Leaf samples from 306 individuals of the three varieties of *Angelica japonica* were collected from 14 populations (Table 2, Fig. 1), and were used for morphological and genetic analyses. The voucher information of the samples is shown in Table 2. All voucher specimens have been deposited at the Herbarium of the Graduate School of Science, Kyoto University (KYO). Identification of the three varieties essentially followed the criteria established by Yamazaki (1990), based on his system of dis-

criminating morphological characteristics (Table 1). However, although Yamazaki identifies the plants from the Hedo population in Okinawa Island as var. *hirsutiflora* due to their ovate to broad-ovate leaflets, we identify them as var. *japonica* because our previous allozyme study clearly shows that the plants of the Hedo population are genetically more similar to those from the Kyushu and Honshu populations, which are recognized to be var. *japonica* (Seo *et al.* 2004).

Observation of leaflet morphology

In order to investigate the relationship between genetic similarity and morphology, we observed two leaflet characteristics, shape and serration of cauline leaflets, following the characteristics described by Yamazaki (1990).

TABLE 2. Populations of the three varieties of *Angelica japonica* studied by allozyme analysis.

Population	Locality	voucher no.	N
var. <i>japonica</i>			
Kushikino	Kushikino city, Kagoshima, Kyushu	Seo 5901-5920	20
Sata	Sata cho, Kagoshima, Kyushu	Seo 5921-5942	22
Hedo	Hedomisaki, Okinawa Island, Ryukyu Islands	Seo 5943-5962	20
var. <i>hirsutiflora</i>			
Motobu	Motobu cho, Okinawa Island, Ryukyu Islands	Seo 5963-5981	19
Arume	Higashi son, Okinawa Island, Ryukyu Islands	Seo 5982-6000	19
Kuroshima	Kuro Island, Ryukyu Islands	Seo 6001-6022	22
Yonaguni	Yonaguni Island, Ryukyu Islands	Seo 6023-6045	23
var. <i>boninensis</i>			
K Daito 1	Kita Daito Island, Daito Islands	Seo 6046-6070	25
K Daito 2	Kita Daito Island, Daito Islands	Seo 6071-6095	25
M Daito 1	Minami Daito Island, Daito Islands	Seo 6096-6120	25
M Daito 2	Minami Daito Island, Daito Islands	Seo 6121-6145	25
Haha 1	Haha Island, Bonin Islands	Nobushima 100001-100022	22
Haha 2	Haha Island, Bonin Islands	Nobushima 100023-100044	22
Haha 3	Haha Island, Bonin Islands	Nobushima 100045-100061	17
total			306

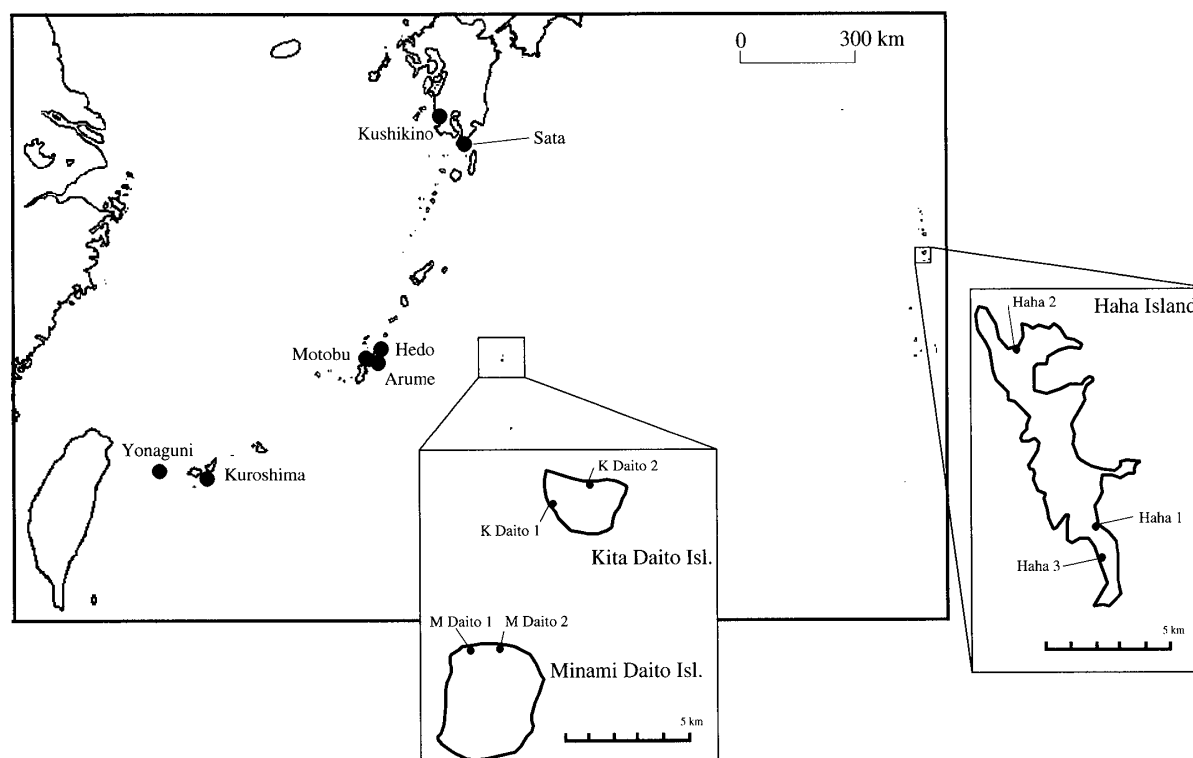


FIG. 1. Locality of *Angelica japonica* populations sampled for allozyme analyses.

Allozyme analysis

About 100 mg of fresh leaf tissue was homogenized in a 1 ml extraction buffer (0.2 % 2-mercaptoethanol, 2 % polyvinyl-pyrrolidone, 0.1 mM Tris-HCl, 1 mM EDTA (4Na), 10 mM KCl, 10 mM MgCl₂, pH = 7.5). After centrifugation of homogenates at 15,000 rpm for 5 minutes at 5 °C, 20 µl of supernatant was used for electrophoresis for each enzyme. Vertical discontinuous polyacrylamide slab gel electrophoresis (Shiraishi 1988) was conducted for genotyping all plant samples. The following 12 enzyme systems were examined: alcohol dehydrogenase (ADH; EC 1.1.1.1), diaphorase (DIA; EC 1.8.1.4), esterase (EST; EC 3.1.1), glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49), glutamate dehydrogenase (GDH; EC 1.4.1.2), glutamate oxaloacetate transaminase (GOT; EC 2.6.1.1), isocitrate dehydrogenase (IDH; EC 1.1.1.42), leucine aminopeptidase (LAP; EC 3.4.11.1), 6-phosphogluconate dehydrogenase (6PG; EC 1.1.1.44), phosphoglucose mutase (PGM; EC 5.4.2.2), phospho-

glucoisomerase (PGI; EC 5.3.1.9), and shikimate dehydrogenase (SKD; EC 1.1.1.25). Staining procedures followed those of Shiraishi (1988) and Richardson *et al.* (1986) with minor modifications.

Loci were numbered in relation to their mobility, with the longest distance zone being isozyme 1, and shorter distance zones were isozyme 2, 3, *etc.* Allele labels denote relative mobility alphabetically, with the *a*-allele of each locus having the longest migrating band.

Data analysis

(1) Genetic variability within populations

The levels of allozyme diversity were estimated for all populations of *Angelica japonica*. Allele frequencies, the proportion of polymorphic loci (*P*), the mean number of alleles per locus (*A*), and the mean expected heterozygosities (*He*) were calculated using POPGENE version 1.31 (Yeh *et al.* 1999).

(2) Genetic differentiation among the populations

Genetic distance and genetic identities among the populations were calculated following Nei (1978), and a UPGMA dendrogram was constructed using PHYLIP version 3.57 (Felsenstein 1995).

Results

Allozyme analysis

The 12 enzyme systems resolved were interpreted as being encoded by the following 16 putative loci: *Adh*, *Dia-1*, *Dia-2*, *Est-1*, *Est-2*, *G6pd*, *Gdh*, *Got-1*, *Got-2*, *Idh*, *Lap*, *6Pg*, *Pgi*, *Pgm-1*, *Pgm-2* and *Skd*. Since *Dia-2*, *Est-1*, *Gdh*, *G6pd*, *6Pg* and *Pgm-2* were observed in an unresolvable staining zone,

they were not scored. Two loci, *Adh* and *Idh*, were monomorphic. The number of alleles per locus ranged from one for *Adh* and *Idh* to four for *Est-2* and *Lap*.

The values of *A*, *P*, and *He* are given in Table 3. The *A*, *P*, and *He* of the Hedo population showed the highest values. At the population level, the genetic diversity of the Ryukyu and Bonin populations was higher than that of the populations of the main island of Japan and the Daito Islands.

The genetic identities and distances among all populations of the three infraspecific taxa are shown in Table 4. A UPGMA dendrogram was obtained based on Nei's genetic distances among all the pop-

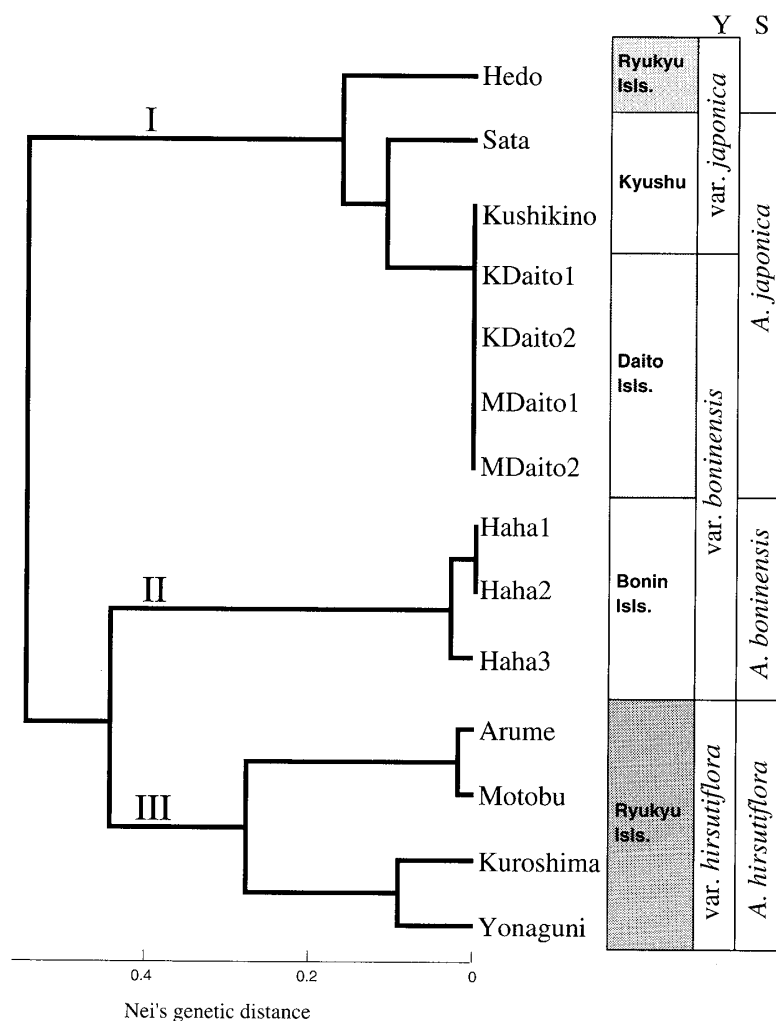


FIG. 2. UPGMA dendrogram of *Angelica japonica* based on Nei's genetic distance. The infraspecific classification (Y) of Yamazaki (1990) and our new classification (S) are indicated in the right margin. The Hedo population is treated as var. *japonica* (see Materials and Methods).

ulations (Fig. 2). Based on the obtained UPGMA dendrogram, three major clusters were recognized. Cluster I includes two populations in the southern part of Kyushu, one in the Hedo population of Okinawa Island, and four populations of the Daito Islands (vars. *japonica* and *boninensis*); cluster II includes three populations of the Bonin Islands (var. *boninensis*); and cluster III includes two populations of Okinawa Island and two populations of

the Yaeyama Islands (var. *hirsutiflora*). The plant populations of the Daito and the Bonin Islands, which were recognized as var. *boninensis* by Yamazaki (1990), were divided into two different clusters based on the UPGMA dendrogram.

Genetic identities between clusters ranged from 0.64 (cluster II vs. cluster III) to 0.55 (cluster I vs. cluster II) (Table 5). These values are lower than the mean values of those found among con-

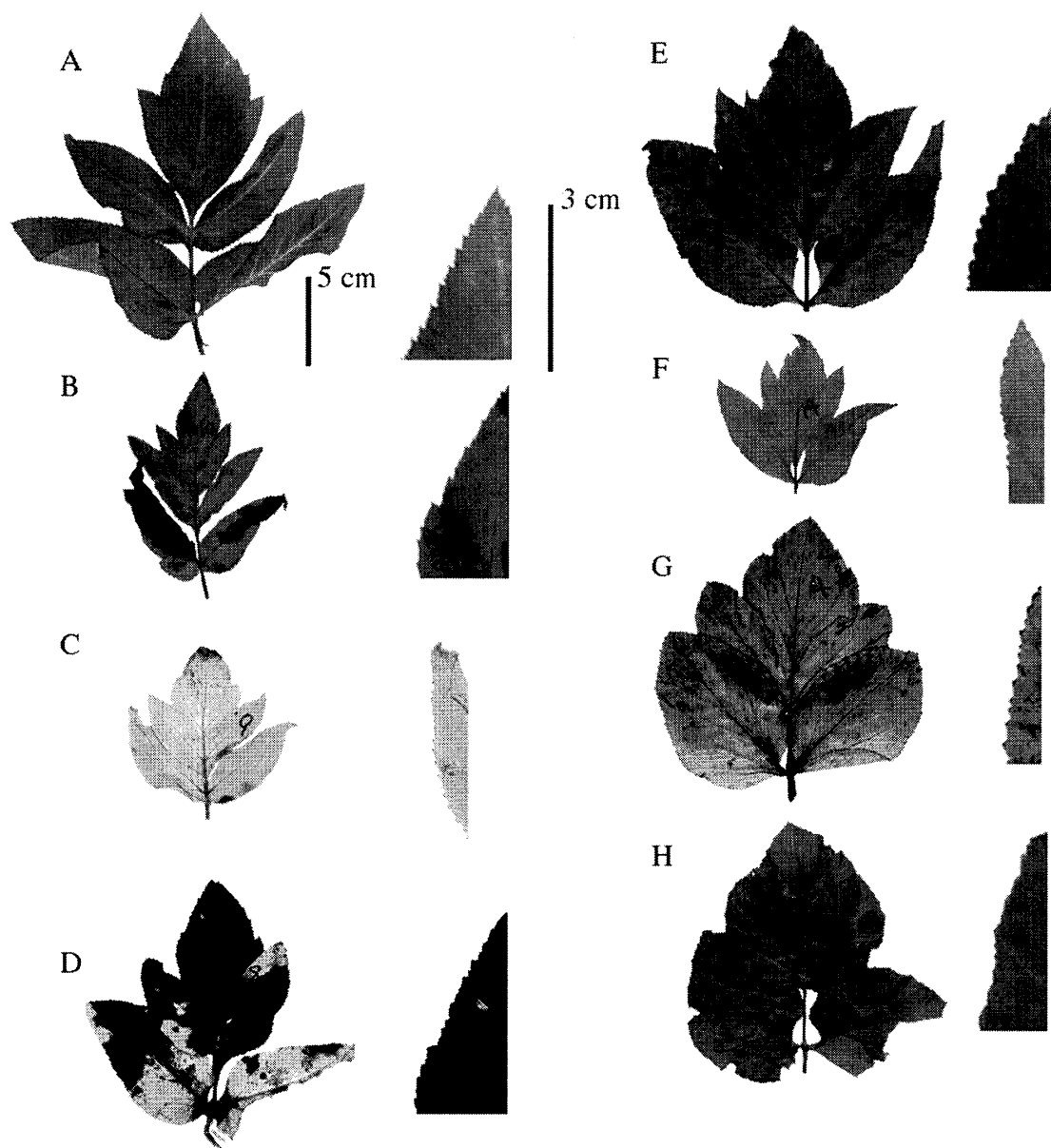


FIG. 3. Terminal leaflets (left) and serration (right) of *Angelica japonica*, *A. hirsutiflora*, and *A. boninensis*. (A) *A. japonica* collected from Kushikino; (B) *A. japonica* from Minami Daito Island; (C) and (D) *A. japonica* from Okinawa Island (Hedomisaki); (E) and (F) *A. boninensis* from Haha Island; (G) *A. hirsutiflora* from Okinawa Island (Motobu); and (H) *A. hirsutiflora* from Yonaguni Island.

TABLE 3. Comparison of genetic variability in populations of *Angelica japonica* based on three measurements: *A*, the mean number of alleles per locus; *P*, the proportion of polymorphic loci; *He*, mean expected heterozygosities.

Population	<i>A</i> (s. d.)	<i>P</i>	<i>He</i> (s. d.)
Kushikino	1.00	0.00	0.00
Sata	1.00	0.00	0.00
Hedo	1.50 (0.70)	0.40	0.13 (0.19)
Motobu	1.10 (0.31)	0.10	0.03 (0.08)
Arume	1.30 (0.48)	0.30	0.10 (0.18)
Kuroshima	1.40 (0.70)	0.30	0.12 (0.21)
Yonaguni	1.20 (0.42)	0.18	0.02 (0.06)
Kita Daito 1	1.00	0.00	0.00
Kita Daito 2	1.00	0.00	0.00
Minami Daito 1	1.00	0.00	0.00
Minami Daito 2	1.00	0.00	0.00
Haha 1	1.10 (0.32)	0.10	0.05 (0.16)
Haha 2	1.10 (0.32)	0.10	0.05 (0.16)
Haha 3	1.20 (0.42)	0.20	0.05 (0.10)

specific plant populations (Crawford 1983).

Leaflet morphology

Three types of leaflet morphology were observed in *Angelica japonica*: i) acutely serrated lower leaves and oblong to ovate-elliptic leaflets (Fig. 3, A-C), ii) obtusely serrated lower leaves and ovate-elliptic leaflets (Fig. 3, E and F), and iii) obtusely serrated lower leaves and widely ovate-triangular leaflets (Fig. 3, D, G, and H). Leaflet shape of types i and iii were observed in Hedo populations (Fig. 3, C and D). All plants in cluster I except those from the Hedo population had type i leaflets. Plants of all Daito populations had type i leaflets and showed no leaflets with an intermediate shape between that of type i and type ii (Fig. 3, B). All plants in clusters II and III had type ii and type iii leaflets, respectively.

Discussion

Three clusters of the populations of *Angelica japonica* were identified based on the obtained UPGMA dendrogram (Fig. 2). However, these clusters did not coincide with the infraspecific taxa identified by Yamazaki (1990). Yamazaki (1990) presented that

the leaflet shape of the plants from the Daito Islands was intermediate between that of var. *japonica* from the main island of Japan or from the Ryukyu Islands, and that of var. *boninensis* from the Bonin Islands. Our observation indicate not only genetic similarity but similarity of leaflet shape, suggesting that the plants from the Daito populations should not be attributed to var. *boninensis* but to var. *japonica*.

Several plant species are known to be distributed both on the Bonin Islands and on the Daito Islands (Hatusima 1975). *Angelica japonica* has been considered to be a species with such a distribution pattern (Yamazaki 1990). However, the results of the present allozyme analysis clearly show that the *Angelica* plants on the Bonin and Daito Islands should be recognized as two independent taxa due to the high genetic distance between these populations, which is comparable to that between different plant species (Crawford 1983).

The three clusters identified on our UPGMA dendrogram were mostly consistent in terms of their leaflet shape, indicating that leaflet shape is a good discriminating characteristic for identifying the three genetic groups of *Angelica japonica*. The plants belonging to cluster I, except those from the Hedo population, had acutely serrated lower leaves and oblong to ovate-elliptic leaflets, whereas those belonging to cluster II had obtusely serrated lower leaves and ovate-elliptic leaflets. The plants belonging to cluster III had obtusely serrated lower leaves and widely ovate-triangular leaflets. Thus, some of the plants from the Hedo population showed the leaflet morphological characteristics of cluster III, although the Hedo population clearly belongs to cluster I on the dendrogram in Fig. 2. We first considered that these findings might suggest that the Hedo population is a mixture of the two genetically differentiated types. Based on our observations of the allozyme alleles found in the Hedo population, especially those of *Got-1* and *Got-2* (Appendix), no evidence was obtained supporting the genetic het-

TABLE 4. Nei's unbiased measures of genetic identity and distance of *Angelica japonica* populations.

		var. <i>japonica</i>			var. <i>hirsutiflora</i>				var. <i>boninensis</i>						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Kushikino	****	0.90	0.86	0.69	0.65	0.48	0.59	1.00	1.00	1.00	1.00	0.57	0.58	0.61
2	Sata	0.11	****	0.83	0.61	0.59	0.44	0.50	0.90	0.90	0.90	0.90	0.57	0.58	0.61
3	Hedo	0.15	0.19	****	0.71	0.72	0.49	0.51	0.86	0.86	0.86	0.86	0.47	0.48	0.51
4	Motobu	0.37	0.49	0.34	****	0.98	0.73	0.79	0.69	0.69	0.69	0.69	0.69	0.69	0.68
5	Arume	0.43	0.52	0.32	0.02	****	0.77	0.76	0.65	0.65	0.65	0.65	0.70	0.71	0.70
6	Kuroshima	0.73	0.82	0.70	0.32	0.27	****	0.91	0.48	0.48	0.48	0.48	0.61	0.62	0.61
7	Yonaguni	0.52	0.69	0.68	0.24	0.28	0.09	****	0.59	0.59	0.59	0.59	0.58	0.59	0.58
8	MDaito1	0.00	0.11	0.15	0.37	0.43	0.73	0.52	****	1.00	1.00	1.00	0.57	0.58	0.61
9	MDaito2	0.00	0.11	0.15	0.37	0.43	0.73	0.52	0.00	****	1.00	1.00	0.56	0.55	0.49
10	KDaito2	0.00	0.11	0.15	0.37	0.43	0.73	0.52	0.00	0.00	****	1.00	0.56	0.55	0.49
11	KDaito1	0.00	0.11	0.15	0.37	0.43	0.73	0.52	0.00	0.00	0.00	****	0.56	0.55	0.49
12	Haha1	0.56	0.56	0.76	0.38	0.36	0.49	0.54	0.56	0.56	0.56	0.56	****	1.00	0.97
13	Haha2	0.55	0.55	0.74	0.36	0.34	0.48	0.53	0.55	0.55	0.55	0.55	0.00	****	0.98
14	Haha3	0.49	0.49	0.67	0.38	0.36	0.50	0.55	0.49	0.49	0.49	0.49	0.03	0.02	****

Nei's genetic identities (above diagonal) and genetic distances (below diagonal).

erogeneity of this population. Thus, we tentatively concluded that only the Hedo population had intrapopulational morphological variation, which is rarely seen in *A. japonica* and its related species. However, the plants of the Hedo population and the other populations of Okinawa Is., which constituted cluster III, shared the same alleles at *Est-2* and *Pgm-1* (Appendix). This may suggest that hybridization between the plants of clusters I and III might have occurred only in the Hedo population in the past. More studies are necessary to elucidate how the Hedo population originated, and to conclude how we should classify the plants in this population.

The value of the genetic identities between clusters I, II, and III (Table 5) were within the range of those found among congeneric plants species (Crawford 1983) as well as those found among the species endemic to the Bonin Islands and their putative ancestor species on the main islands of Japan

TABLE 5. Average genetic identity among three clusters of *Angelica japonica*. The values in parentheses indicated the standard deviation.

	I	II	III
I	0.93 (0.07)	0.55 (0.04)	0.59 (0.09)
II		0.98 (0.01)	0.64 (0.05)
III			0.82 (0.10)

and the Ryukyu Islands (Ito 1998). The three Bonin populations were found to share endemic alleles at *Est-2* and formed an independent cluster. The low genetic identities among the three clusters on the UPGMA dendrogram suggest that these clusters should be treated as independent species, specifically, as *Angelica japonica* A. Gray (cluster I), *A. boninensis* Tuyama (cluster II), and *A. hirsutiflora* Liu, Chao et Chung (cluster III). The plants in these three clusters can be usually identified by the morphological characteristics mentioned above, which further support the present taxonomical treatment.

Taxonomical note

Key to species of *Angelica japonica*, *hirsutiflora*, and *boninensis*

- 1a. Serrations of radical and lower cauline leaves acute. Rarely, serrations of radical and lower cauline leaves obtuse *A. japonica*
- 1b. Serrations of radical and lower cauline leaves obtuse
 - 2a. Terminal leaflets widely ovate-triangular; pedicels densely hairy ... *A. hirsutiflora*
 - 2b. Terminal leaflets obtuse; pedicels glabrescent or sparsely hairy *A. boninensis*

Angelica japonica A. Gray, Mem. Amer. Acad. II. 6: 39, (1859); Hiroe & Constance, Umbelliferae of Japan, 116 (1958), p. p. excl. loc. Ins. Yoron, austr. Ins. Okinawa, Ins. Sakishima; Ohwi, Flora of Japan, 683 (1965), p. p. excl. loc. Ins. Yoron, austr. Ins. Okinawa, Ins. Sakishima.

Angelica Sieboldi Miquel, Ann. Mus. Lugd.-Bat. 3: 61(1867). Not *Peucedanum Sieboldi* Miquel, 1867. Based upon a specimen presumably from Japan, collected by Siebold.

Angelica kiusiana Maxim., Bull. Acad. St. Petersb. 18: 284 (1873). Based upon Maximowicz, RHab. in Japoniae ins. Kiusiu: litore arenoso sinum Simabarae circa pagum Himi, non procul a Nagasaki.S

Stout, 1-1.5 m tall, the foliage puberulent on the nerves of the upper surface, glaucous beneath, the inflorescence villous; radical and lower cauline leaves petiolate; leaves deltoid, 20-50 cm long, ternately-pinnately divided, the leaflets oblong to ovate-elliptic, rarely widely ovate-triangular, 7-15 cm long, 3-7 cm broad, acute to obtuse, mostly sessile, mucronate-serrate, rarely obtuse-serrate; petioles stout, 20-50 cm long, sheathing; cauline leaves reduced upward, with moderately dilated sheaths; peduncles stout, 6-30 cm long; involucre wanting; rays 20-40, 2-8 cm long, spreading-ascending, subequal; involucre of several broadly linear to lanceolate, scarious, villous bractlets 13-15 mm long, mostly shorter than the flowers; pedicels 2-15 mm long, spreading-ascending; flowers white, the petals obovate, glabrous; styles slender, twice as long as the conic stylopodium; ovaries essentially glabrous; fruit oblong-oval, 6-10 mm long, 5-7 mm broad, the dorsal ribs very narrowly winged, the lateral broader than the dorsal and about equaling the body; vittae usually several in the intervals, 2 on the commissure; seed face slightly concave.

Type locality: Kakeroma Island, Kagoshima Prefecture, Japan. "obtained only at Katanosima, one of the islands between Japan and the proper Loo

Choo groups" [Ryukyu Islands], C. Wright (Lectotype).

Distribution: South-central Honshu to Kyushu; Osumi Isls, Amami Isl., Kikai Isl. Tokunoshima Isl., Okinoerabu Isl., Daito Isls, southern part of Korea.

Japanese name: Hamaudo.

Specimens examined: HONSHU. **Niigata:** Katsugi, Iwahune-gun, *G. Koidzumi s.n.*, May 5, 1929 (KYO). **Shizuoka:** Gozenzaki, *Y. Kurozawa s.n.*, May 4, 1930 (KYO); Suzaki, *S. Kitamura 154* (KYO). **Aichi:** Irakozaki, Atsumi-gun, *M. Hiroe 16080* (KYO). **Toyama:** Abujima, *T. Ota s.n.*, Aug. 2, 1930 (KYO). **Ishikawa:** Hegurajima Isl., *Yoshikawa s.n.*, Aug. 3, 1929 (KYO). **Fukui:** Tojinbo, *Z. Tashiro s.n.*, May 15, 1929 (KYO). **Mie:** Mt. Hiyori, Shima-gun, *M. Hiroe 19040* (KYO); Wagu to Momotori, Toshi Isl., Toba-shi, *G. Murata 20676* (KYO). **Kyoto:** Kanmuri Isl., *G. Murata 67763* (KYO), *S. Tsugaru & T. Takahashi 29362, 28107* (KYO); Ohnyu-Chitose, Maidzuru-shi, *S. Tsugaru & T. Takahashi 18695* (KYO); Yosa-gun, *Y. Araki 9714* (KYO); Kutsushima Isl., Maidzuru-shi, *S. Tsugaru & T. Sawada 30770* (KYO). **Wakayama:** Tsubaki, Higashitonda-mura, *G. Murata 5767* (KYO); Oshima, *Z. Tashiro s.n.*, Aug. 10, 1934 (KYO), *I. Namikawa s.n.*, June 21, 1940 (KYO). **Hyogo:** Akaho, Akaho-gun, *M. Hiroe 7803* (KYO); Ikishima Isl., Akaho-gun, *M. Hiroe 7325* (KYO); Sumoto-shi, Narugashima Isl., *T. Fukuhara 7230* (KYO); Nu-shima Isl., Mihara-gun, *N. Fukuoka & N. Kurosaki 11457* (KYO). **Yamaguchi:** Mt. Kasa-yama, Hagi-shi, *M. Hotta 5957* (KYO); Sasazaki, Kitaurakaigan, *I. Hino & K. Oka 5028* (KYO). Shikoku. Kagawa: Kannonji-shi, *Z. Tashiro s.n.*, June 14, 1930 (KYO). SHIKOKU. **Ehime:** Nishiuwa-gun, *T. Jinbo s.n.*, May 5, 1951 (KYO); Minamiuwa-gun, *G. Koidzumi s.n.*, Aug. 15, 1934 (KYO). KYUSHU. **Nagasaki:** en route from Toyo to Toorazuham, Kamiagata-gun, Tsushima Isl., *N. Fukuoka 7856* (KYO). **Kagoshima:** Shibushi, *G. Koidzumi s.n.*, Nov. 11, 1917 (KYO); Satamisaki, *G. Koidzumi s.n.*, Nov. 6, 1917 (KYO); Oodomari to Tajiri, Sata-cho, *A. Nitta 45* (KYO); Along Onna-gawa, Yaku Isl. *T. Yahara, J. Murata & H. Ohba 9009* (KYO); Amami Isl., *G. Koidzumi s.n.*, Apr., 1923 (KYO), *G. Koidzumi s.n.*, May 1, 1923 (KYO); Kasari, Amami Isl., *S. Tsugaru 402* (KYO); Tekutsuku, Kikai Isl., *K. Mimoro, T. Shimada & T. Ozaki 2723* (KYO); Wadamari, Okierabu Isl., *J. Murata 4805* (KYO).

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Angelica japonica var. *hirsutiflora* (T. S. Liu, C. Y. Chao et T. I. Chuang) T. Yamazaki. Journ. Jap. Bot. 65: 222 (1990).

The leaflets widely ovate-triangular, obtuse-serrate; pedicels densely hairy; wings of fruits thick.

Type locality: Shimen, Taipei Hsien, Taiwan. *T. I. Chuang* 3979 (TAI).

Distribution: Yoron Isl., Okinawa Isls., Miyako Isls., Yaeyama Isls., Taiwan.

Japanese name: Nangoku-Hamaudo.

Specimens examined: **Okinawa**: Nakagami, Okinawa Isl., *G. Koidzumi s.n.*, May 15, 1923 (KYO); Naha, Okinawa Isl., *G. Koidzumi s.n.*, May 15, 1923 (KYO), *Z. Tashiro s.n.*, Apr. 1, 1931 (KYO); Oota-Fukai, Ishigaki Isl., *N. Fukuoka & N. Kurosaki* 12095 (KYO); Hirara, Miyako Isl., *G. Koidzumi s.n.*, June 11, 1923 (KYO); Yonaguni Isl., *G. Koidzumi s.n.*, July 11-13, 1923 (KYO); Yonehara, Ishigaki Isl., *H. Okada, A. Takahashi & H. Nagamasu* 393 (KYO); Funaura-Nonara, Iriomote Isl., *N. Fukuoka* 11420 (KYO); Hateruma Isl., *H. Okada & K. Ueda* 2045 (KYO). Taiwan. Shih-men, Taipei Hsien, *K. Y. Yen & M. T. Kao* 5134 (KYO).

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Peucedanum boninensis (Tuyama) Tuyama, Bot. Mag. Tokyo 50: 430. (1936); Hara, Enum. Sperm. Jap. 3: 317 (1954); Toyoda, Fl. Bonin Is., 193, f. 87 (1981).

The leaflets obtuse, obtuse-serrate; pedicels glabrescent or sparsely hairy; wings of fruits thin.

Type locality: Hirashima Isl., *T. Tuyama s.n.*, Apr. 10, 1934 (TI).

Distribution: Endemic to the Ogasawara Isls. Endemic.

Japanese name: Munin-Hamaudo.

Specimens examined: Tokyo: Chichijima Isl., *Y. Momiyama, S. Kobayashi & M. Ono* 125960 (KYO), *N. Fujita & Y. Shimizu* 62 (KYO); Hirashima Isl., *T. Tuyama s.n.*, Apr. 10, 1934 (KYO); Nakoudojima Isl., *T. Tuyama s.n.*, Apr. 10, 1936 (KYO).

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APPENDIX Allele frequencies of *Angelica japonica* populations.

	var. <i>japonica</i>			var. <i>hirsutiflora</i>				var. <i>boninensis</i>						
	Kushikino	Sata	Hedomisaki	Motobu	Arume	Kuroshima	Yonaguni	KDaito 1	KDaito 2	MDaito 1	MDaito 2	Haha 1	Haha 2	Haha 3
<i>Dia-1</i> a			0.08			1.00	1.00							
b	1.00	1.00	0.93	1.00	1.00			1.00	1.00	1.00	1.00	1.00	1.00	1.00
<i>Est-2</i> a			0.75	0.25	0.47	0.61								
b		1.00					0.04							
c	1.00		0.25	0.75	0.53	0.39	0.96	1.00	1.00	1.00	1.00			
d												1.00	1.00	1.00
<i>Got-1</i> a				1.00	1.00	1.00	1.00					1.00	1.00	0.53
b	1.00	1.00	1.00					1.00	1.00	1.00	1.00			
d														0.47
<i>Got-2</i> a				1.00	1.00	1.00	1.00					1.00	1.00	0.53
b	1.00	1.00	1.00					1.00	1.00	1.00	1.00			
c														0.47
<i>Lap</i> a					0.29	0.61	0.13							
b														
c												1.00	1.00	1.00
d	1.00	1.00	1.00	1.00	0.71	0.12	0.87	1.00	1.00	1.00	1.00			
e						0.27								
<i>Pgi</i> a			0.53		0.16				0.50					
b			0.05											
c	1.00	1.00	0.43	1.00	0.84	1.00	1.00	1.00	0.50	1.00	1.00	1.00	1.00	1.00
<i>Pgm-1</i> a			0.70	1.00	1.00									
b	1.00	1.00	0.30					1.00	1.00	1.00	1.00	1.00	1.00	1.00
c						1.00	1.00							
<i>Skd</i> a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.57	0.66	0.88
b												0.43	0.34	0.12